Germination of the teleutospores of Ravenelia cassiæcola.

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(WITH PLATES IX AND X.)

As far as can be ascertained the publications relative to the morphology of the genus *Ravenelia* have yet given no idea of the germination of the teleutosporic stage. From results of anatomical studies in 1886, Parker[1] concludes that the structure of teleutospores is really that of a cluster of fused teleutosporic stalks. Cunningham[2] gives an interesting exposition of the development of the successive forms in two East Indian species, and also traces the development of teleutospores. He shows that the cysts are essentially modified basal cells of the true spore cells, and their origin is illustrated. He makes clearer the relation borne to other members of the group of Uredineæ. However, his attempts at artificial cultivation of teleutospores proved failures,[3] and he is not positive as to the success of experiments relative to the artificial infection.

In the biological laboratory of the Alabama Polytechnic Institute, and under the direction of Prof. Geo. F. Atkinson, artificial cultures have been made with some successful results. Inasmuch as the designation teleutospore involves the idea of the production of promycelia and sporidia, we can expect results to be only of generic importance.

Since the genus is not as well known as its relatives, it may be well to observe some of the specific characters of *R. cassiæcola* Atkinson,[4] which I draw largely from the author's description. The teleutosporic form occurs most abundantly on the stems of the host plant, *Cassia nictitans*; yet it also attacks pods and leaves. The sori are usually irregularly oblong and very dark in color. The teleutospores are more or less brown, composed of from three to thirty somewhat wedge-shaped cells, the width of the head being usually from 50 to 90μ. The compound colored pedicel is from 10 to 18μ wide, and the length generally about 80μ, yet it may be twice

[1] Morphology of *Ravenelia glandulæformis*. Proc. Am. Acad. of Arts and Sci., vol. XXII.

[2] Notes on the life history of *R. sessilis* B. and *R. stictica* B. & Br. Scientific Memoirs by Medical Officers of the Army of India.

[3] Cooke, Journ. Royal Mic. Soc., vol. III, p. 389, says: "The utmost that we have been able to accomplish has been to obtain single short germinating threads from the apices of a few of the pseudospores in *R. aculeifera*."

[4] Described in BOTANICAL GAZETTE, Nov., 1891, as "A new Ravenelia from Alabama."

as long. At the junction of the pedicel with the spore cells we observe the characteristic cyst cells. These are hyaline or slightly colored, usually spherical, and average about 12 μ in diameter (for normal spores see figs. 14 and 15).

After remaining in water for some time, maceration of the spores is to a certain extent effected, and by slight pressure the individual cells are easily separable for examination. It is then apparent that *R. cassiæcola*, so far as the arrangement of cells is concerned, belongs to the division as noted by Parker of which *R. Indica* is the type; consequently the cells are only laterally coherent. Besides the close union of adjacent cells, there is an external coat common to all which holds them together more firmly. In thickness, this is less than the exterior walls of individual cells (fig. 16), and from its surface often appear short, hyaline or slightly colored spines.

Specimens of the host plant containing the fungus in good condition were collected early in December, after the death of the vegetation. Water cultures, both slide and cell, then manifested no germination after being observed for a number of days. To continue the preservation of the material as in a natural state, it was simply "heeled in" under a box in an exposed place, and it was from this material that results were finally obtained. Slide and cell cultures with both distilled water and sugar solution were failures early in January, but on Feb. 23 the first results were secured. This occurred only in the sugar solution, and nine days after the spores had been sown. The results here given are from the same. It may be of interest to note that well-dried herbarium specimens have germinated after remaining three weeks in a cell culture with water.

The promycelia issue about perpendicularly to the plane of cells, bending towards the upper surface in an abundance of the medium, and when the spore lies on its side. Until a little greater than the length of the head, they are observed to be simple tubes completely filled with protoplasmic contents. These tubes rapidly elongate, the protoplasm collecting in the distal extremity, and they become finally from two to five times the length of the head (figs. 1 and 2). A promycelium may become branched, usually when meeting an obstruction to its growth, and this may be nearly at right angles to the former course (fig. 3). In all cases, however, the protoplasm remains separated only a short time, rapidly

collecting in the growing extremity. Apparent septa are sometimes observed (fig. 4 *a* and fig. 6 *a*), but the deception results from a coherence of granular contents in a cross section of the tube; and by moving towards the point of growth, this protoplasm soon mingles with the mass at the normal location (fig. 7 *a*). Variations of the above may be found in the empty spaces sometimes noticeable (fig. 13, *a*, etc.), and these are most abundant after a considerable growth has taken place. Small vacuoles are not infrequent.

The sporidia are developed at or very near the terminal portion of the promycelia (fig. 4, *a* and *b*). The first evidence of this formation is shown by a part of the protoplasm collecting into a side branch, whose connecting portion is but little smaller than the main tube, and which assumes more or less the usual characters of a sterigma. With this development of sporidia a more highly refractive power is manifest. A sporidium measures about 9 μ in diameter, but its form is not generally spherical. In most cases the abscised reproductive body shows a prolongation at the end by which it was attached (fig. 5 *a* and *b*), the constriction which eventually sets the body free encroaching somewhat on the usual limits of the sterigma in the group of Uredineæ. Vacuoles are frequently present, but these vary in number and in size.

Sporidia are not always produced, and their absence is counterbalanced by a longer growth of the tubes. This greater growth probably results from the fact that the promycelia are completely immersed in water. Lagerheim[5], speaking of the germination in water of *Puccinia heterogena* Lagerheim, says, "They then germinate exactly like uredospores; a long non-septate germ tube, often bent backward and forward, and with a strongly undulating contour, grows out of the germ pore. . . . Probably the fungus can reproduce itself by these germ tubes, which, because they form no sporidia, penetrate directly into the leaf." It is possible that under favorable conditions the same may be true for the long promycelial growths of the fungus we are considering. A promycelium often shows an enlargement at the end, as if a terminal sporidium were to be produced, but instead, the tube may be again normally continued. With the above character, a geniculation is often noticeable, the new growth

[5] Journal of Mycology, vol. VII, no. 1.

resulting in the protrusion of the wall in an oblique direction; or the latter character may exist independently of the former. A peculiar instance is shown in fig. 9, *a* and *b*, where a sporidium seems to be almost fully developed laterally, then its wall is protruded from near its base into a new tube which again branches. It seems that a promycelium bears only one of these reproductive bodies, as more than that number have not been observed; still this cannot now be positively asserted. The true development of the promycelia is often interfered with on account of parasitic attacks. Owing to the large size of the spores of this fungus, they carry many adhering germs into the cultures, and the tender promycelial tubes are favorite spots for bacterial growth. It appears that the germination of a sporidium is by the prolongation of the pedicel-like end of attachment (fig. 5, *c*).

If the cells of a germinating teleutospore are separated by gentle pressure under a cover glass, the emergence of the promycelia from the germ pores can be noted. In the peripheral cells, which are externally somewhat convex, the germ pore is situated at the upper and inner extremity (fig. 10), while in the more angular central cells it may be at any distal corner (fig. 12, *a*). In all cases it is marked by the junction of the thick external cell wall with the thinner wall separating individual cells. The germ pore can be more distinctly seen by the usual examination with sulphuric acid.

In Ravenelia cassiæcola only has the germination of teleutospores thus far been observed, but these notes serve to indicate that the germination is generically characteristic. It differs from that of such typical genera as Puccinia, etc., in the non-septate character of the promycelia, except in such species as P. heterogena above mentioned, where the germination takes place in an abundance of water. "In *Coleosporium*," says Plowright,[6] "each cell produces a single promycelial spore," and from his illustration of *C. senecionis* we observe that the tube which bears this sporidium tapers gradually to a very small size. Now if we deem both promycelium and sterigma essential terms, it is difficult to differentiate their limits in such cases. Sorauer[7] only states that each cell develops a simple promycelium with a sporidium. De Bary[8] defines

[6] British Uredineæ and Ustilagineæ, p. 45.
[7] Pflanzenkrankheiten, 2te Aufl., II, p. 244.
[8] Morphology and Biology of the Fungi, etc., p. 281.

the character of producing a single sporidium as peculiar to Coleosporium, but he names the entire tube from which this body is abscised a sterigma. Since the term sterigma is more or less broad, we may regard *Coleosporium* as possessing a truly non-septate promycelium, and still the above details will perhaps make clear the essential modifications in R. cassiæcola and probably the general features in the genus Ravenelia.

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Explanation of Plates IX and X.

Plate IX.—Fig. 1, germinating teleutospore, showing normal condition of promycelium. Fig. 2, same as above with a slight geniculation and rudimentary branching. Fig. 3, a promycelial branch almost at right angles to the former course. Fig. 4, *a* and *b*, stages in the development of sporidia; *a* also shows apparent septa. Fig. 5, *a* and *b*, sporidia; *c*, sporidium germinating. Fig. 6, *a* and *b*, abnormal conditions of promycelia. Fig. 7, *a* and *b*, same as fig. 6, but representing appearances on following day. Fig. 8, teleutospore with single promycelium and abscised sporidium germinating while still in the vicinity of its point of production.

Plate X.—Fig. 9, *a* and *b*, peculiar development of a promycelium noted on successive days. Figs. 10, 11 and 12, representing cells separated by pressure, and showing the location of the germ pores and the emergence of the promycelia. Fig. 13, teleutospore germinating, but so surrounded by other spores that the promycelia are modified. Figs. 14 and 15, normal teleutospores of different number of cells. Figs. 16, *a*, *b* and *c*, individual cells, showing relative thickness of cell walls and the common external coat.

All figures were drawn under camera lucida.

Notes on Carex. XVI.

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An unusual amount of carex material has come into my hands within the last year, bringing a number of new species, extending the ranges of well known species to an important extent, and affording data for the clearing up of old doubts. These specimens have come from almost every part of North America and from very many collectors; in fact, the carex flora of the country has never had so many friends as at present. Some of the most important facts concerning the geographical distribution of species are recorded below.

Carex obesa All., var. *minor* Boott, heretofore not known south of Saskatchewan, was collected last July upon high bluffs at South Fowl Lake, Northern Minnesota, by F. F. Wood.